

Technical Procedures for Processing with Diaminobenzidine

1 Scope

Diaminobenzidine is used by FBI Laboratory Friction Ridge Discipline personnel to develop latent prints and enhance visible prints deposited in blood. The process can be used on all surfaces but is primarily used on porous items.

2 Equipment/Materials/Reagents

Beakers or graduated cylinders

Balance

Spatula

Distilled water3,3'-Diaminobenzidine Tetrahydrochloride (purity $\geq 97\%$)

Hydrogen Peroxide (30% Solution)

1M Phosphate Buffer Solution (pH 7.4)

5-Sulfosalicylic Acid (purity $\geq 99\%$)

3 Procedures

3.1 Solution Preparation

Personnel will prepare the solutions as follows. Alternative amounts may be prepared, provided the same ratio of chemicals mixed is retained.

3.1.1 Solution A (Fixer solution)

Combine:

- 5-Sulfosalicylic Acid - 20 g
- Distilled water - 1000 ml

Stir until 5-Sulfosalicylic Acid dissolves.

3.1.2 Solution B (Buffer solution)

Combine:

- 1 M Phosphate Buffer Solution - 100 ml
- Distilled water - 800 ml

3.1.3 Solution C (Diaminobenzidine solution)

Combine:

- 3,3'-Diaminobenzidine Tetrahydrochloride - 1 g
- Distilled water - 100 ml

Stir until 3,3'-Diaminobenzidine Tetrahydrochloride dissolves.

3.1.4 Developer solution

Combine:

- Solution B (Buffer solution) - 180 ml
- Solution C (Diaminobenzidine solution) - 20 ml
- Hydrogen Peroxide (30% solution) - 1 ml

Mix thoroughly.

3.2 Application

Personnel will complete the following steps in order:

1. Apply Solution A (Fixer solution) to item by spraying, dipping, squirting, painting, or tissue method. Leave on item for 3 to 5 minutes.
2. Apply distilled water to item by spraying, dipping, squirting, painting, or tissue method. Leave on item for 30 to 60 seconds.
3. Apply Developer solution to item by spraying, dipping, squirting, painting, or tissue method. Leave on item to achieve maximum contrast and development but do not exceed 5 minutes.
4. Apply distilled water to item by spraying, dipping, squirting, painting, or tissue method to stop development process.
5. Allow item to dry.

For digital capture and photography, see FBI Friction Ridge Discipline Processing Manual Preamble.

3.3 Storage of Solutions

Solution A (Fixer solution) and Solution B (Buffer solution) may be stored in any type of laboratory accepted receptacle.

Solution C (Diaminobenzidine solution) must be stored frozen in a container that can withstand extreme cold.

Storage is not applicable to developer solution. It is prepared as needed.

3.4 Shelf Life

Solution A (Fixer solution) and Solution B (Buffer solution) have indefinite shelf lives provided the reagent checks are satisfactory.

Solution C (Diaminobenzidine solution) has a 6 month shelf life when frozen provided the reagent checks are satisfactory.

Developer solution is not retained and is prepared as needed.

4 Standards and Controls

See FBI Friction Ridge Discipline Processing Manual, Preamble.

5 Safety

See FBI Laboratory Safety Manual for appropriate information.

6 Sampling

Not applicable.

7 Calculations

Not applicable.

8 Measurement Uncertainty

Not applicable.

9 Limitations

Not applicable.

10 References

Allman, D. and Pounds, C. A. "Diaminobenzidine: A Simple, Safe, and Sensitive Method for the Enhancement of Blood Marks at a Scene of Crime and in the Laboratory". *CRSE Report*. No. 733.

FBI Laboratory Safety Manual, Federal Bureau of Investigation, Laboratory Division. Latest Revision.

FBI Friction Ridge Discipline Processing Manual, Preamble, Federal Bureau of Investigation, Laboratory Division. Latest Revision.

Trozzi, T. A., Schwartz, R. L., and Hollars, M. L. *Processing Guide for Developing Latent Prints*, FBI Laboratory, Washington DC, 2001.

Rev. #	Issue Date	History
1	10/02/17	Specific section numbers referenced in Preamble removed throughout document. Section 1, latent print personnel added. Section 4 removed and remaining renumbered. Titles for Section 4 and Section 7 modified. Section 9, generalized. Updated for Biometrics Analysis Unit. References updated. Abbreviations addressed.
2	07/15/21	Replace Latent Print Units with Friction Ridge Discipline. Minor wording changes. Term “specimen” changed to “item” throughout. Streamline equipment list. Re-organization and re-numbering of sections. Section 1, added last sentence. Section 3.1 separated out into Section 3.1.1, Section 3.1.2, Section 3.1.3, and Section 3.1.4 and added ratio allowances for solutions. Section 4, added Preamble reference.

Approval

Redact - Signatures on File

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Date: 07/14/2021

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